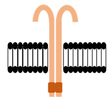
Characterization of metabolic gene expression in the RAW 264.7 cell line

Lauren Macaisa and Judith D. Ochrietor
Department of Biology, University of North Florida, Jacksonville, Florida USA

Introduction

The innate immune response is the first line of defense against pathogens. The response is non-specific in that patterns rather than specific molecules, are detected. White blood cells, including monocytes, express cell-surface proteins that detect pathogens and initiate an immune response.

Toll-like receptors (TLRs) are a major group of pattern recognition receptors (PRRs) expressed on the surface of a variety of cells that function to recognize molecular patterns associated with all classes of pathogenic microorganisms (reviewed by Kawasaki et al., 2014). TLR4 specifically recognizes the lipopolysaccharide (LPS) component of Gram-negative bacterial cell walls.

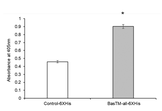


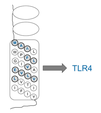
TLR4 has leucine-rich repeats in the extracellular domain, a single-pass transmembrane domain, and a toll-interferon receptor (TIR) domain in the cytoplasmic tail

Basigin (CD147) is a member of the immunoglobulin superfamily (IgSF) that functions as a cell adhesion molecule. It has been implicated in a variety of processes, including reproduction and development, metabolism, and cancer (reviewed by Muramatsu, 2016).

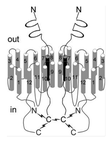
Basigin (CD147) has two extracellular Ig domains, a single-pass transmembrane domain, and a short cytoplasmic tail

A recent study by this laboratory suggests that TLR4 and Basigin interact via their transmembrane domains (Brown, 2016, UNF Graduate thesis).

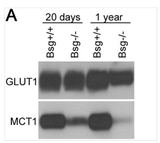




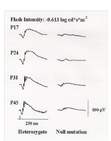
Studies of the neural retina indicate that Basigin gene expression is linked to expression of Monocarboxylate Transporter 1 (MCT1), which moves lactate, pyruvate, and ketone bodies across the plasma membrane via facilitated diffusion (Philp et al., 2003; Halestrap and Price, 1999). Basigin-null mice do not express Basigin or MCT1 on photoreceptors or Müller cells, which impairs delivery of metabolites from the Müller cells to the photoreceptor cells (Philp et al., 2003). The result is blindness in Basigin-null mice from the time of eye opening (Ochrietor et al., 2002).



Wilson et al., 2002



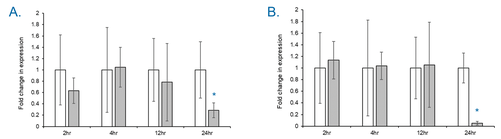
Philp et al., 2003



Ochrietor et al., 2002

Purpose / Hypothesis

A recent study by this laboratory showed that the expression of Basigin and MCT1 transcripts is significantly decreased in monocytes treated with LPS for 24 hours.



Expression of Basigin (A) and MCT1 (B) transcripts in RAW 264.7 cells. The open bars represent D-PBS-treatment and the gray bars represent LPS-treatment. The error bars represent the Coefficient of Variation.
*=p<0.05 via T-test

The purpose of the present study was to determine if MCT1 protein expression is also decreased after 24 hours of exposure to LPS. Additionally, the expression of the glucose transporter GLUT1 was assessed.

It was hypothesized that MCT1 proteins would be decreased in LPS-treated cells, when compared to the D-PBS treated cells. Additionally, it was hypothesized that the decrease in MCT1 expression would result in the concomitant increase in GLUT1 expression to indicate a metabolic shift in response to the inflammatory stimulus.

Methodology

RAW 264.7 monocytes were grown at 37°C in 5% CO_2 in RPMI 1640 medium (Gibco – Life Technologies, Grand Island, NY) containing 10% FBS (HyClone, GE Healthcare-BioSciences, Marlborough, MA).

RAW 264.7 cells were plated in serum-free RPMI 1640 medium (Gibco – Life Technologies) and allowed to attach.

The cells were treated with LPS (1 μg/mL; InvivoGen, San Diego, CA) or Dulbecco's phosphate buffered saline (D-PBS; Gibco – Life Technologies) for 24 hours at 37°C in 5% CO_2.

The cells were subjected to immunocytochemistry (Tokar et al., 2017) using antibodies specific for MCT1 and GLUT1 (Millipore Corporation, Billerica, MA). The cells were visualized on an Olympus FV1000 microscope and fluorescence was quantified using the accompanying software. Expression in LPS-treated cells versus D-PBS-treated cells was compared using a paired, one-tailed T test.

Results

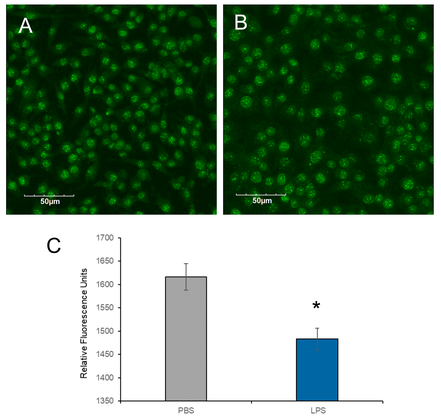


Figure 1. Expression of MCT1 in RAW 264.7 cells as measured by immunocytochemistry. Images of D-PBS treated (A) and LPS treated (B) RAW 264.7 cells are shown. The magnification bar is equal to 50 μm. (C) The relative fluorescence units corresponds to the amount of MCT1 expression in each group at 24 hours of treatment. The gray bars represent D-PBS-treatment and the blue bars represent LPS-treatment. The values represent the average of 50 regions of interest (ROIs) and the error bars represent the standard error of the mean. * = p<0.05 via T-test.

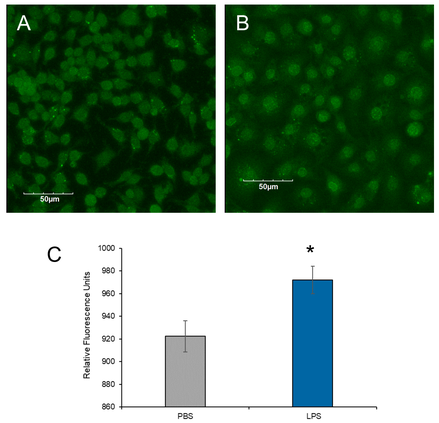


Figure 2. Expression of GLUT1 in RAW 264.7 cells as measured by immunocytochemistry. Images of D-PBS treated (A) and LPS treated (B) RAW 264.7 cells are shown. The magnification bar is equal to 50 μm. (C) The relative fluorescence units corresponds to the amount of MCT1 expression in each group at 24 hours of treatment. The gray bars represent D-PBS-treatment and the blue bars represent LPS-treatment. The values represent the average of 50 regions of interest (ROIs) and the error bars represent the standard error of the mean. * = p<0.05 via T-test.

Results

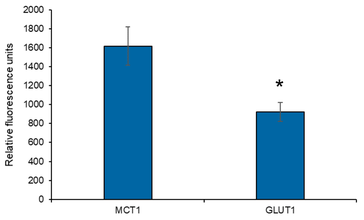


Figure 3. Expression of MCT1 and GLUT1 in RAW 264.7 cells as measured by immunocytochemistry. The fluorescence units corresponds to the level of expression of each protein in RAW 246.7 cells incubated in D-PBS. The values represent the average of 50 regions of interest (ROIs) and the error bars represent the standard error of the mean. * = p<0.05 via T-test.

Conclusions and Comments

Although it was initially hypothesized that metabolic responses would increase in monocytes to support immune mechanisms, the data for MCT1 suggest that metabolism is reduced in activated monocytes. To determine if the decrease in MCT1 expression indicates a shift of resources from general cellular maintenance to induction of an innate immune response, the expression of the glucose transporter GLUT1 was also investigated.

The expression of MCT1 is much greater than that of GLUT1. as measured by immunohistochemistry. This implies that monocarboxylates like pyruvate, lactate, and ketone bodies are transported into circulating monocytes to a greater degree than glucose.

The expression of GLUT1 did increase in monocytes in response to LPS treatment. This suggests that monocytes shift their metabolic resources during an immune response to use glucose more prevalently than monocarboxylates.

Acknowledgements

The authors thank the UNF Office of Academic Affairs for their financial support of this project.

The authors also thank Dr. Terri N. Ellis, UNF Department of Biology, for the generous gift of RAW 264.7 cells.